B28

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/705, 14/34, 14/235, 14/445, 14/165, 14/77, 14/12, 14/135, 14/11, 14/62, 14/02, 14/09, 14/145, 16/28, 16/08, 16 /10, 16 /12, 16 /18, 16 /20, 16 /26, A61K 39 /00, 39 /015, 39 /05, 39 /10, 39 /145, 39 /135, 39 /165, 39 /205, 39 /29, 39 /39

A1

(11) International Publication Number: WO 95/23166

(43) International Publication Date: 31 August 1995 (31.08.95)

(21) International Application Number: PCT/AU95/00090

(22) International Filing Date: 24 February 1995 (24.02.95)

(30) Priority Data:
PM 4119 25 February 1994 (25.02.94) AU

(71) Applicant (*for all designated States except US*): DEAKIN RESEARCH LIMITED [AU/AU]; Level 1, 80 Mount Street, North Sydney, NSW 2060 (AU).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): COMIS, Alfio [AU/AU]; 3 Prairie Vale Road, Bossley Park, NSW 2176 (AU). TYLER, Margaret, Isabel [AU/AU]; 43A Trentino Road, Turramurra, NSW 2074 (AU). FISCHER, Peter [CH/NO]; Nycomed Bioreg A/S, Gaustadalleen 21, N-0371 Oslo (NO).

(74) Agent: GRIFFITH HACK & CO.; G.P.O. Box 4164, Sydney, NSW 2001 (AU).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: SYNTHETIC INVERSO OR RETRO-INVERSO T-CELL EPITOPES

(57) Abstract

Synthetic T cell epitope analogues of native T cell epitopes which are partially or completely inverso or retro-inverso modified with respect to the native T cell epitope are shown to be effective as T cell epitopes. These T cell epitope analogues stimulate immune responsiveness when used in place of their native T cell epitope counterparts in vaccines. The invention further relates to the use of these T cell epitope analogues, to vaccines comprising the T cell epitope analogues, to methods of preparing vaccines comprising these T cell epitope analogues, and to antibodies generated using these T cell epitope analogues.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

SYNTHETIC INVERSO OR RETRO-INVERSO T-CELL EPITOPES

TECHNICAL FIELD

The present invention relates to synthetic T cell epitope analogues of native T cell epitopes with partial or complete inverso or retro-inverso modifications. These T cell epitope analogues stimulate immune responsiveness when used in place of their native T cell epitope counterparts in vaccines. The invention further relates to the use of these T cell epitope analogues, to vaccines comprising the T cell epitope analogues, to methods of preparing vaccines comprising these T cell epitope analogues, and to antibodies generated using these T cell epitope analogues.

BACKGROUND ART

The stereochemistry of polypeptides can be described in terms of the topochemical arrangement of the side chains of the amino acid residues about the polypeptide backbone which is defined by the peptide bonds between the amino acid residues and the α-carbon atoms of the bonded residues. In addition, polypeptide backbones have distinct termini and thus direction.

The majority of naturally occurring amino acids are L-amino acids. Naturally occurring polypeptides are largely comprised of L-amino acids.

D-amino acids are the enantiomers of L-amino acids and form peptides which are herein referred to as inverso peptides, that is, peptides corresponding to native peptides but made up of D-amino acids rather than L-amino acids.

Retro-inverso modification of naturally occurring polypeptides involves the synthetic assemblage of amino acids with α-carbon stereochemistry opposite to that of the corresponding L-amino acids, i.e. D- or D-allo-amino acids, in reverse order with respect to the native peptide sequence. A retro-inverso analogue thus has reversed termini and reversed direction of peptide bonds

while approximately maintaining the topology of the side chains as in the native peptide sequence.

Partial retro-inverso peptide analogues are polypeptides in which only part of the sequence is reversed and replaced with enantiomeric amino acid residues. Since the retro-inverted portion of such an analogue has reversed amino and carboxyl termini, the amino acid residues flanking the retro-inverted portion are replaced by side-chain-analogous α-substituted geminal-diaminomethanes and malonates, respectively.

Processes for synthesis of retro-inverso peptide analogues (Bonelli *et al.*, 1984; Verdini and Viscomi, 1985) and some processes for the solid-phase synthesis of partial retro-inverso peptide analogues have been described (Pessi *et al.*, 1987).

It has been observed that due to the stereospecificity of enzymes with respect to their substrates, replacement of L-amino acid residues with D-amino acid residues in peptide substrates generally abolishes proteolytic enzyme recognition and/or activity, although exceptions are known.

Peptide hormones have been of particular interest as targets for retro-inversion, presumably because their analogues would have potential use as therapeutic agents. Partial, and in a few cases complete, retro-inverso analogues of a number of peptide hormones have been prepared and tested (see, for example, Goodman and Chorev, 1981).

Complete or extended partial retro-inverso analogues have generally been found to be devoid of biological activity. The lack of biological activity has been attributed to possible complex structural changes caused by extended modification, the presence of reversed chain termini or the presence of proline residues in the sequences. Some partial retro-inverso analogues, that is peptides in which only selected residues were modified, on the other hand, have been shown to retain or enhance biological activity. Retro-inversion has also found

application in the area of rational design of enzyme inhibitors.

The fact that retro-inversion of biologically active peptides has met with only limited success in retaining or enhancing the activity of the native peptide is probably due to several reasons. Although structurally very similar, it was realised early that peptides and their retro-enantiomers are topologically not identical and crystal structure and solution conformation studies have borne this out. Biological activity of a peptide hormone or neurotransmitter depends primarily on its dynamic interaction with a receptor, as well as on transduction processes of the peptide-receptor complex. It is now clear that such interactions are complex processes involving multiple conformational and topological properties. Consequently it is not surprising that a retro-inverso analogue may not be able to mimic all of these properties.

In order to activate the cellular component of the immune system a vaccine must present T-cell epitopes, as well as pathogen-specific B-cell epitopes. T cells fail to recognise soluble antigen. They require its presentation on the surface of antigen presenting cells (APC) in association with molecules encoded by the major histocompatibility complex (MHC). In the case of large proteins which constitute conventional vaccines, the protein undergoes enzymatic digestion intracellularly. Some of the resulting peptide fragments can bind to MHC molecules and the peptide-MHC complexes are then transported to the surface of APCs. The peptides capable of binding MHC molecules are T-cell epitopes. Because of the genetic restriction of the MHC, the sequences which can act as T-cell epitopes may vary amongst individuals in an outbred population. Totally synthetic vaccines (Jolivet *et al.*, 1990) therefore need to be designed with regard to these facts. While it is possible to provide T-cell epitopes in a peptide vaccine by conjugation of the relevant B-cell epitope peptides to a carrier protein

such as tetanus toxoid, this is not desirable because it negates the inherent advantages of a peptide vaccine, *e.g.* chemical stability and ease of production. The identification of appropriate T-cell epitope 'cocktails' potentially useful in synthetic vaccines is therefore an active field of research (Schwartz, 1986).

DISCLOSURE OF THE INVENTION

Definitions

Throughout the specification and claims "retro modified" refers to a peptide which is made up of L-amino acids in which the amino acid residues are assembled in opposite direction to the native peptide with respect to which it is retro modified.

Throughout the specification and claims "inverso modified" refers to a peptide which is made up of D-amino acids in which the amino acid residues are assembled in the same direction as the native peptide with respect to which it is inverso modified.

Throughout the specification and claims "retro-inverso modified" refers to a peptide which is made up of D-amino acids in which the amino acid residues are assembled in the opposite direction to the native peptide with respect to which it is retro-inverso modified.

Throughout the specification and claims the term "native" refers to any sequence of L amino acids used as a starting sequence for the preparation of partial or complete retro, inverso or retro-inverso analogues.

The term "peptide" as used throughout the specification and claims is to be understood to include peptides of any length.

Throughout the specification and claims the term "antigenic fragment" refers to a peptide which is a portion of an antigen which itself is immunogenic or capable of binding antibodies.

The term "antigen" as used throughout the specification and claims is to be understood to include immunogens as the context requires.

Throughout the specification and claims the term "antigen analogue" refers to a peptide molecule capable of mimicking the immunological activity of the native peptide antigen with respect to which it is partially or completely retro, inverso or retro-inverso modified. Retro peptides are made up of L-amino acids and are peptides in which the amino acid residues are assembled in opposite direction to the native peptide sequence.

Throughout the specification and claims the term "T-cell epitope analogue" refers to a peptide molecule capable of mimicking the immunological activity of the native T-cell epitope with respect to which it is partially or completely inverso or retro-inverso modified.

Partial modification includes analogues in which as few as two consecutive residues are modified. Typically at least 5 or 6 consecutive residues are modified.

The present invention relates to partially or completely inverso or retro-inverso modified T-cell epitope analogues of native T cell epitopes which stimulate immune responsiveness when used in place of their native T cell epitope counterparts in vaccines. Incorporation of D-amino acids into T-cell epitope analogues increases their stability to degradation after administration. Further, incorporation of D-amino acids has potential for oral administration of analogues.

Having shown that particular retro-inverso or inverso T-cell epitope analogues can stimulate immune responsiveness when used in the place of their native T-cell epitope counterparts it follows that, generally, these analogues can be expected to be successful since T-cell epitope - MHC molecule interactions are not fundamentally different from case to case.

In a first aspect the invention provides a synthetic peptide T cell epitope analogue of a native T cell epitope, which analogue is partially or completely inverso or retro-inverso modified with respect to the native T cell epitope.

The T cell epitope analogues of the present invention stimulate immune responsiveness when used in place of their native T cell epitope counterparts in vaccines.

The efficacy of T cell epitope analogues of the invention is illustrated with respect to the malaria T cell epitopes of Example 2.

In a second aspect the invention provides a vaccine comprising a T cell epitope analogue of the first aspect together with a B cell epitope and a pharmaceutically or veterinarally acceptable carrier, diluent, excipient and/or adjuvant. Typically, the vaccines of the invention are cocktails of T cell epitope analogues and B cell epitopes tailored to the condition against which vaccination is required. Preferably the T cell epitope analogue is conjugated to the B cell epitope.

The B cell epitope is conjugated to the T cell epitope by standard chemical conjugation techniques or the conjugate is synthesized as a continuous peptide.

The B cell epitope can be provided as any epitope, or any intact molecule providing the epitope, against which an antibody response is required.

The B cell epitopes to be incorporated into vaccines in accordance with the invention include peptides or polypeptides of any length whose amino acid sequences stem from polypeptides of pathogens such as poliomyelitis, hepatitis B, foot and mouth disease of livestock, tetanus, pertussis, HIV, cholera, malaria, influenza, rabies or diphtheria causing agents, or toxins such as robustoxin, heat labile toxin of pathogenic *Escherichia coli* strains and Shiga toxin from *Shigella dysenteriae*. Other B cell epitopes of interest include epitopes of Amyloid ß protein (Alzheimer's disease) and human chorionic gonadotropin and gonadotropin releasing hormone (contraceptive vaccines).

The B cell epitope is preferably a retro, retro-inverso or inverso antigen analogue.

Preferred T cell epitope analogues of the invention

are analogues of:

Diphtheria toxin:

H-Gln-Val-Val-His-Asn-Ser-Tyr-Asn-Arg-Pro-Ala-Tyr-Ser-Pro-Gly-OH (SEQ ID NO: 1)

Pertussis toxin:

H-His-Arg-Met-Gln-Glu-Ala-Val-Glu-Ala-Glu-Arg-Ala-Gly-Arg-OH (SEQ ID NO: 2)

Malaria CSA protein:

H-Pro-Ser-Asp-Lys-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-OH (SEQ ID NO: 3)

Malaria CSB protein:

H-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-OH (SEQ ID NO: 4)

Malaria CST3 protein:

H-Gly-Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser-OH (SEQ ID NO: 5)

Hen egg lysozyme:

H-Cys-Ser-Ala-Leu-Leu-Ser-Ser-Asp-Ile-Thr-Ala-Ser-Val-Asn-Cys-Ala-OH (SEQ ID NO:6)

Ovalbumin:

H-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-OH (SEQ ID NO: 7) and

H-Tyr-Thr-Tyr-Thr-Val-His-Ala-Ala-His-Ala-Tyr-Thr-Tyr-Thr-OH (SEQ ID NO: 8)

Other preferred T cell epitope analogues are analogues of:

Measles Virus F and H glycoproteins: (Partidos C.D. *et al*, 1991)

MVF:258-277	H-Gly-Ile-Leu-Glu-Ser-Arg-Gly-Ile-Lys-Ala-Arg-Ile-Thr-His-Val-Asp-Thr-Glu-Ser-Tyr-OH (SEQ ID NO: 9)
MVF:288-302	H-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-OH (SEQ ID NO: 10)

Respiratory syncytial virus 1A protein: (Nicholas J.A. *et al*, 1988)

RS1A:45-60	H-Cys-Glu-Tyr-Asn-Val-Phe-His-Asn-Lys-Thr-Phe-Glu-Leu-Pro-Arg-Ala-OH (SEQ ID NO: 11)

Influenza hamagglutinin A/PR/8/34 Mt.S.:

109-119	(Hackett C.J. *et al* 1983)	(SEQ ID NO: 12)
130-140	(Hurwitz J.J. *et al* 1984)	(SEQ ID NO: 13)
302-313	(Lamb J.R. *et al* 1982; Hurwitz J.L. *et al* 1984)	(SEQ ID NO: 14)
Pork Insulin:		
(A)4-14	(Rosenthal A.S. 1978)	(SEQ ID NO: 15)
(B)5-16	(Thomas J.W. *et al* 1981)	(SEQ ID NO: 16)
Hepatitis B virus pre S:		
120-132	(Milich D.R. *et al* 1986)	(SEQ ID NO: 17)
Hepatitis B virus major surface antigen:		
38-52	(Milich D.R. *et al* 1985)	(SEQ ID NO: 18)
95-109	"	(SEQ ID NO: 19)
140-154	"	(SEQ ID NO: 20)
Foot and mouth virus VP1:		
141-160	(Francis M.J. *et al* 1985)	(SEQ ID NO: 21)
Rabies virus-spike glycoprotein precursor:		
32-44	(Macfarlan R.I. *et al* 1984)	(SEQ ID NO: 22)

In a third aspect the invention provides a method of vaccinating a host in need of such treatment which method comprises administering an effective amount of a vaccine according to the second aspect to the host.

In a fourth aspect the invention provides antibodies produced by immunisation of a host with a vaccine of the second aspect.

In a fifth aspect the invention provides a method of preparing a T cell epitope analogue of the invention comprising synthesising a partially or completely inverso or retro-inverso peptide comprising the analogue.

In a sixth aspect the invention provides a method of preparing a vaccine of the second aspect comprising conjugating a T cell epitope analogue of the first aspect to a B cell epitope or admixing a T cell epitope analogue of the first aspect with a B cell epitope and admixing an effective amount of the resulting mixture or conjugate with a pharmaceutically or veterinarally acceptable carrier, diluent, excipient and/or adjuvant.

Vaccines of the invention can be formulated using

standard methods in the art of vaccine formulation.

Selection of appropriate diluents, carriers, excipients and/or adjuvants can be made in accordance with standard techniques in the art.

Vaccines of the invention may be administered to hosts in need of such treatment by injection. Vaccines incorporating D-amino acid containing analogues may also be administered orally.

ABBREVIATIONS

BOP	(benzotriazolyloxy)tris(dimethylamino) phosphonium hexafluorophosphate (Castro's reagent)
DMF	dimethyl formamide
ELISA	enzyme-linked immunosorbent assay
Fmoc	9-fluorenylmethoxycarbonyl
HPLC	high-performance liquid chromatography
Ig	immunoglobulin
in	inverso
i.p.	intraperitoneal
no	normal (native)
PBS	phosphate buffered saline (10 mM phosphate, 150mM NaCl, pH 7.4)
Pfp	pentafluorophenyl
PVC	polyvinylchloride
ri	retro-inverso
TFA	trifluoroacetic acid

Amino Acids:

L-amino acids are indicated by an upper case followed by lower case lettering e.g. Ala indicates L-alanine.

D-amino acids are indicated by all lower case abbreviations, e.g. ala indicates D-alanine.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of a cell proliferation experiment conducted using the T-cell epitope peptides

noMalCST3 (SEQ ID NO: 5), inMalCST3 and riMalCST3.

Figure 2 shows antibody production measured in mice immunized with the B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) alone or together with either no or riMalCST3.

Figure 3 shows antibody production measured in mice immunized with the B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) alone or together with either no (SEQ ID NO: 3) or riMalCSA protein.

Figure 4 shows antibody production measured in mice immunized with the B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) alone or together with either no (SEQ ID NO: 4) or riMalCSB protein.

Figure 5 shows antibody production measured in mice immunized with the B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) alone or together with either no (SEQ ID NO: 1) or riDiphT.

Figure 6 shows antibody production measured in mice immunized with the B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) alone or together with either no (SEQ ID NO: 2) or riPertT.

Figure 7 shows antibody production measured in mice immunized with the B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) alone or together with either no (SEQ ID NO: 7) or riOvalT.

BEST MODE OF CARRYING OUT THE INVENTION

T cell epitope analogues of the invention are prepared by standard techniques for the preparation of L and D amino acid containing peptides, particularly as outlined in Example 1.

Vaccines of the invention are formulated by standard techniques for vaccine formulation using standard carriers, diluents excipients and/or adjuvants suitable for the formulation of oral or injectable vaccines. Effective amounts of Tcell-epitope analogues to be incorporated in the vaccines can be determined in accordance with standard methods. Conjugation techniques

where used are standard chemical conjugation techniques.

The vaccination regimes used are standard regimes for the vaccination of animal or human hosts. These regimes can be used where immunisation of the host is desired or where the host is being used to produce antibodies for exogenous use.

The invention is further described in the following examples which are illustrative of the invention but in no way limiting on its scope.

EXAMPLE 1

Peptide Synthesis

Peptides were synthesised by a solid-phase method on polyamide (Arshady *et al.*, 1981) or Polyhipe supports using side-chain protected Fmoc amino acids (Carpino & Han, 1972), essentially as described by Eberle *et al.* (1986). Only pure amino acid derivatives, obtained commercially or by synthesis, were used. The polyamide synthesis resins, derivatised with p-alkoxybenzyl alcohol-based linkage agents, were esterified quantitatively with the appropriate preformed C-terminal Fmoc-amino acid symmetrical anhydrides, in the presence of 0.2 molar equivalents of N,N-dimethylaminopyridine and N-methylmorpholine. The Polyhipe resin, derivatised with Fmoc-Rink linker (Rink, 1987) did not require esterification of the first amino acid linked to it. Chain elongation was carried out using Fmoc-amino acid pentafluorophenyl esters (Atherton *et al.*, 1988) or Castro's reagent/1-hydroxybenzotriazole coupling (Hudson, 1988). The progress of each synthesis was monitored using a specific colour test (Hancock & Battersby, 1976) and/or amino acid analysis of acid-hydrolysed peptidyl resin samples.

The peptides were cleaved from the resins and side-chain deprotected with the aid of TFA, containing a suitable mixture of scavenger chemicals (Tam, 1988). After filtration and vacuum evaporation, the peptides were triturated with diethyl ether, collected by

centrifugation and lyophilised from aqueous ammonium bicarbonate solution.

All peptides then underwent an initial desalting and purification step by column chromatography on suitable gel filtration media in aqueous solvents. Afterwards they were purified to homogeneity by reversed-phase HPLC using water-acetonitrile (containing 0.05-0.1% TFA) gradient elution. The purity of the synthetic peptides was further assessed by gas-phase acid hydrolysis/amino acid analysis (Bidlingmeyer *et al.*, 1987) and, if deemed necessary, by automated gas-phase sequencing (Hunkapiller & Hood, 1983).

EXAMPLE 2

Malaria T-cell epitope peptides

It has been shown that nonresponsiveness to the malaria immunodominant B-cell epitope $(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_x$ (SEQ ID NO: 23) of the *Plasmodium falciparum* circumsporozoite protein can be overcome in the presence of a particular T-cell epitope peptide from the same protein (Sinigaglia *et al*, 1988). The peptide in question, unlike most T-cell epitopes, is recognised in association with most human MHC class II molecules and has been suggested as a suitable component of a synthetic peptide vaccine against malaria. The region of the circumsporozoite protein from which the peptide stems is apparently conserved in different parasite isolates.

The following peptides were prepared according to the usual protocols:

noMalCST3	H-Gly-Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser-OH (SEQ ID NO: 5)
inMalCST3	H-Gly-asp-ile-glu-lys-lys-ile-ala-lys-met-glu-lys-ala-ser-ser-val-phe-asn-val-val-asn-ser-OH
riMalCST3	H-ser-asn-val-val-asn-phe-val-ser-ser-ala-lys-glu-met-lys-ala-aile-lys-lys-glu-aile-asp-Gly-OH

BALB/c mice were immunised subcutaneously at the base of the tail with the above T-cell epitope peptides emulsified in an equal volume of complete Freund's adjuvant. Ten days later, the animals were killed by cervical dislocation and the inguinal and popliteal lymph nodes removed. A cell suspension from the lymph nodes was prepared and the cells cultured in the presence of various concentrations of the test antigen, as well as a non-related control antigen. Cell proliferation was quantitated by measuring the incorporation into the cells of radiolabelled thymidine. Results from the experiment are shown in Fig. 1.

When animals were primed with any form of the peptide and the animals' cultured T cells challenged with the same peptide, proliferation was observed in every case. Upon priming with one form of a peptide and challenging with either of the other two forms, some activation was observed in each case.

In order to remove any potential effects due to non-specific cell proliferation, the T cell assay method was improved as follows:

A cell suspension from the lymph nodes was centrifuged on Ficoll-Isopaque to separate mononuclear cells from erythrocytes. The resulting cell preparation was washed extensively in PBS and incubated with Dynabeads coated with anti-mouse IgG to remove B-lymphocytes. The cells from this preparation were then cultured in the presence of various concentrations of the test antigen, as well as a non-related control antigen. Cell proliferation was quantitated by measuring the incorporation into the cells of radiolabelled thymidine and or by the use of Promega Cell Titer 96 AQ kit. Again efficacy of the T cell epitope analogues was demonstrated.

Antibody responses to synthetic peptides representing the immunodominant B-cell epitope H-(Asn-Ala-Asn-Pro)$_3$-OH (SEQ ID NO: 23) of the circumsporozoite protein were measured following intraperitoneal injection

of Balb/c mice. One hundred microgrammes of B-cell epitope were administered in an equal volume of Freund's complete adjuvant either alone or in a mixture (1:1) with either noMalCST3 (SEQ ID NO: 5) or riMalCST3. As a negative control, a further group of mice were immunised with either noMalCST3 (SEQ ID NO: 5) or riMalCST3 in the absence of the B-cell epitope. Three weeks after priming, mice were boosted by the same route and with the same dose of peptide in incomplete Freund's adjuvant. A second booster injection was given two weeks after the first with the same dose of antigen in incomplete Freund's adjuvant. Blood samples were taken five days later by retro-ocular bleeding and, after centrifugation, the sera was immediately used in an enzyme-linked immunosorbent assay (ELISA). Titres of antibodies against the B-cell epitope were determined in microtitre plates coated overnight at 4°C with 0.5 microgrammes of synthetic peptide cross-linked to ovalbumin.

Low titre of antibodies were measured in mice immunised with the B-cell epitope alone, however, much higher titre of antibodies was observed in each case in mice co-immunised with the same peptide and either form of the T-cell epitope (Fig.2). All together, these findings demonstrate the potential usefulness of riMalCST3 and inMalCST3 as vaccine components; the cellular immune response they elicit is responsive to the normal antigen.

Antibody response to the same B-cell epitope was also measured using five more T-cell epitopes selected from the literature and synthesized in the following forms:

<u>Malaria circumsporozoite protein:</u>

noMalCSA (Good *et al*, 1987):

H-Pro-Ser-Asp-Lys-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-NH_2 (SEQ ID NO: 3)

riMalCSA:

H-ser-ile-ser-asn-lys-ile-lys-lys-leu-tyr-gln-glu-ile-his-lys-asp-ser-pro-NH_2

noMalCSB (Good *et al*, 1988):
H-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-NH_2 (SEQ ID NO: 4)
riMalCSB:
H-ser-ile-ser-asn-lys-ile-lys-lys-leu-tyr-gln-glu-ile-his-NH_2
Diphtheria toxin:
noDipT (Bixler *et al*, 1989)
H-Gln-Val-Val-His-Asn-Ser-Tyr-Asn-Arg-Pro-Ala-Tyr-Ser-Pro-Gly-NH_2 (SEQ ID NO:1)
riDipT:
H-Gly-pro-ser-tyr-ala-pro-arg-asn-tyr-ser-asn-his-val-val-gln-NH_2
Pertussis toxin:
noPertT (Kim *et al*, 1990) (SEQ ID NO: 2):
H-His-Arg-Met-Gln-Glu-Ala-Val-Glu-Ala-Glu-Arg-Ala-Gly-Arg-NH_2
riPertT:
H-arg-Gly-ala-arg-glu-ala-glu-val-ala-glu-gln-met-arg-his-NH_2
Ovalbumin:
noOvalT (Sette *et al*, 1989) (SEQ ID NO: 7):
H-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-NH_2
riOvalT:
H-glu-asn-ile-glu-ala-his-ala-ala-his-val-ala-gln-ser-ile-NH_2

The synthesis of the above peptides was performed on Polyhipe Rink resin. The side chain protecting groups used were: t-butyl for serine, threonine, aspartic acid, glutamic acid and tyrosine; trityl for histidine, glutamine and asparagine; t-butoxycarbonyl for lysine and 2,2,5,7,8-pentamethyl chroman-6-sulphonyl for arginine. For diphtheria and pertussis peptides, cleavage and side-chain deprotection were accomplished by reaction of the peptidyl resins for 90 min at 0°C with 1M trimethylsilylbromide-thioanisole in TFA containing 0.25M 1,2-ethanedithiol (5% v/v) and water (5% v/v) in TFA at

room temperature for 90 min.

In each case the mice developed very low titres against the B-cell epitope when immunised with the B-cell epitope alone, but produced much higher antibody titre when a mixture of the B-cell epitope and any of the T-cell epitopes in either no- or ri- form were used in the immunogen formulation (Fig. 3-7).

INDUSTRIAL APPLICATION

T cell epitope analogues in accordance with the invention have a range of potential applications in eliciting immunogenic responses in a host. These analogues can be used in the treatment and/or prophylaxis of diseases, and therapy of disease states. In particular, these analogues can be used in vaccines in animals, including humans for protection against pathogens and the like.

REFERENCES

Arshady, R., Atherton, E., Clive, D.L.J. & Sheppard, R.C. (1981) Peptide synthesis. Part 1. Preparation and use of polar supports based on poly(dimethylacrylamide). J. Chem. Soc. Perkin Trans. I, 529-537.

Atherton. E., Cameron, L.R. & Sheppard, R.C. (1988) Peptide synthesis. Part 10. Use of pentafluorophenyl esters of fluorenylmethoxycarbonylamino acids in solid phase peptide synthesis. Tetrahedron, 44, 843-857.

Bidlingmeyer, B.A., Tarvin, T.L. & Cohen, S.A. (1987) Amino acid analysis of submicrogram hydrolyzate samples. In "Methods in Protein Sequence Analysis", Walsh, K.A. (Ed.), pp. 229-245, The Humana Press.

Bonelli, F., Pessi, A. & Verdini, A.S. (1984) Solid phase synthesis of retro-inverso peptide analogues. Int. J. Peptide Protein Res., 24, 553-556.

Carpino, L.A. & Han, G.Y. (1972) The 9-fluorenylmethoxy-carbonyl amino-protecting group. J. Org. Chem., 37, 3404-3409.

Eberle, A.N., Atherton, E., Dryland, A. & Sheppard, R.C. (1986) Peptide synthesis. Part 9. Solid-phase synthesis of melanin concentrating hormone using a continuous-flow polyamide method. J. Chem. Soc. Perkin Trans I, 361-367.

Goodman, M. & Chorev, M. (1981) The synthesis and confirmational analysis of retro-inverso analogues of biologically active molecules. In 'Perspectives in Peptide Chemistry'; Karger, Basel; pp. 283-294.

Hancock, W.S. & Battersby, J.E. (1976) A new micro-test for the detection of incomplete coupling reactions in solid-phase peptide synthesis using 2,4,6-trinitrobenzene-sulphonic acid. Anal. Biochem., 71, 260-264.

Hudson, D. (1988) Methodological implications of simultaneous solid-phase peptide synthesis. 1. Comparison of different coupling procedures. J. Org. Chem., 53, 617-624.

Hunkapillar, M.W. & Hood, L.E. (1983) Protein sequence analysis: automated microsequencing. Science, 219, 650-659.

Pessi, A., Pinori, M., Verdini, A.S. & Viscomi, G.C. (1987) Totally solid phase synthesis of peptide(s) - containing retro-inverted peptide bond, using crosslinked sarcosinyl copolymer as support. European Patent 97994-B, 30 Sep. 1987 (8739).

Tam, J.P. (1988) Acid deprotection reactions in peptide synthesis. In 'Macromolecular Sequencing and Synthesis, Selected Methods and Application', pp. 153-184; Alan R. Liss, Inc.

Verdini, A.S. & Viscomi, G.C. (1985) Synthesis, resolution, and assignment of configuration of potent hypotensive retro-inverso bradykinin potentiating peptide 5a(BPP5a) analogues. J. Chem. Soc. Perkin Trans. I, 697-701.

H. Rink (1987) Solid-phase synthesis of protected peptide fragments using a trialkoxy-diphenyl-methylester resin. Tetrahedron Lett., 28,3787-3790

B.J. Spalding (1992) In hot pursuit of an HIV vaccine. Bio/Technology, 10, 24-29

R.A. Wirtz, J.F. Duncan, E.K. Njelesoni, I. Schneider, A.E. Brown, C.N. Oster, JBO Were and H.K. Webster (1989) Bull WHO, 67,535-542. ELISA method for detecting *Plasmodium falciparum* circumsporozoite antibody.

Steward, M.W. & Howard, C.R. (1987) Synthetic peptides: a next generation of vaccines? Immunol. Today, 8, 51-58.

M. Jolivet, L. Lise, H. Gras-Masse, A. Tartar, F. Audibert & L. Chedid (1990) Polyvalent synthetic vaccines: relationship between T epitopes and immunogenicity. *Vaccine*, 8,35-40.

R.H. Schwartz (1986) The value of synthetic peptides as vaccines for eliciting T-cell immunity. *Current Topics Microbiol. Immunol.*, 130, 79-84.

F. Sinigaglia, M. Guttinger, J. Kilgus, D.M. Doran, H. Matile, H. Etlinger, A. Trzeciak, D. Gillessen & J.R.L. Pink (1988) A malaria T-cell epitope recognized in

association with most human MHC class II molecules. *Nature*, 336, 778-780.

M.F. Good, W.L. Maloy, M.N. Lunde, H. Margalit, J.L. Cornette, G.L. Smith, B. Moss, L.H. Miller & J.A. Berzofsky (1987) Construction of synthetic immunogen: use of new T-helper epitope on malaria circumsporozoite protein. *Science*, 235, 1058-1062.

M.F. Good, D. Pombo, D.L. Maloy, V.F. De La Cruz, L.H. Miller & J.A. Berzofsky (1988) Parasite polymorphism present within minimal T cell epitopes of *Plasmodium falciparum* circumsporozoite protein. *J. Immunol.*, 140, 1645-1650.

G. Bixler, S. Pillai & R. Insel (1989) T-cell epitope as carriers molecule for conjugate vaccines. WO 89/06974.

K.J. Kim, S. McKinness & C.R. Manclark (1990) Determination of T cell epitopes on the S1 subunit of pertussis toxin. *J. Immunol.*, 144, 3529-3534.

A. Sette, A. Lamont, S. Buus, S.M. Colon, C. Miles & H.M. Grey (1989) Effect of conformational propensity of peptide antigens in their interaction with HMC class II molecules. Failure to document the importance of regular secondary structure. *J. Immunol.*, 143, 1268-1273.

M.J. Francis, C.M. Fry, D.J. Rowlands, F. Brown, J.L. Bittle, R. Houghten & R.A. Lerner (1985) Immunological priming with synthetic peptides of foot and mouth disease virus. *J. Gen. Virol.* 66, 2347.

C.J. Hackett, B. Dietzschold, W. Herhard, B. Ghrist, R. Knorr, D. Gillessen & F. Melchers (1983) Influenza virus site recognized by a murine helper T cell specific for H1 strains. *J. Exp. Med.* 158, 294.

J.L. Hurwitz, E. Heber-Katz, C.J. Hackett & W.J. Gerhard (1984) Characterization of the murine T_H response to influenza virus hemaglutinin: evidence for three major specificities. *Immunol* 133, 3371.

J.R. Lamb, D.D. Eckels, P. Lake, J.N. Woody & N. Green (1982) Human T cell clones recognize chemically synthesized peptides of influenza hemagglutinin. *Nature* 300, 66.

R.I. Macfarlan, B. Dietzschold, T.J. Wiktor, M. Kiel, R. Houghten, R.A. Lerner, J.G. Sutcliffe & H. Koprowski (1984) T cell responses to cleaved rabies glycoprotein and to synthetic peptides. *J. Immunol.* 133, 2748.

D.R. Milich, D.L. Peterson, G.G. Leroux-Roels, R.A. Lerner & F.V. Chisari (1985) Genetic regulation of the immune response to hepatitis B surface antigen (HBsAg). VI. Fine specificity. *J. Immunol.* 134, 4203.

D.R. Milich, G.B. Thornton, A. McLachlan, M.K. McNamara & F.V. Chisari (1986) T and B cell recognition of native and synthetic pre-S region determinants on HBsAg. In *Modern Approaches to Vaccines*. R. Chanock, R.A. Lerner and F Brown, eds. Cold Spring Harbor Laboratories, New York.

J.A. Nicholas, M.A. Mitchell, M.E. Levely, K.L. Rubino, J.H. Kinner, N.K. Harn & C.W. Smith (1988) Mapping an antibody binding site and a T cell stimulating site on the 1A protein of respiratory syncytial virus. *J. Virol.* 62, 4465-4473.

C.D. Partidos, C.M. Stanley & M.W. Steward (1991) Immune responses in mice following immunization with chimeric synthetic peptides representing B and T cell epitopes of measles virus proteins. *J. gen. Vir.* 72, 1293-1299.

A.S. Rosenthal (1978) Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* 40, 146.

J.W. Thomas, W. Danho, E. Bullesbach, J. Fohles & A.S. Rosenthal (1981) Immune response gene control of determinant selection. III. Polypeptide fragments of insulin are differentially recognized by T but not by B cells in insulin immune guinea pigs. *J. Immunol.* 126, 1095.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Deakin Research Limited, N/A N/A
Comis, Alfio
Fischer, Peter
Tyler, Margaret I

(ii) TITLE OF INVENTION: EPITOPES

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Griffith Hack & Co
(B) STREET: Level 8, 168 Walker Street
(C) CITY: North sydney
(D) STATE: New South Wales
(E) COUNTRY: Australia
(F) ZIP: 2060

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: AU PM 4119
(B) FILING DATE: 25-FEB-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kurts, Ann D
(B) REGISTRATION NUMBER: N/A
(C) REFERENCE/DOCKET NUMBER: P21192

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 61 2 957 5944
(B) TELEFAX: 61 2 957 6288
(C) TELEX: AA26547

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Corynebacterium diphtheriae

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser
1               5                   10
Pro Gly
    15
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bordetella pertussis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
His Arg Met Gln Glu Ala Val Glu Ala Glu Arg Ala Gly
1               5                   10
Arg
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Plasmodium falciparum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Pro Ser Asp Lys His Ile Glu Gln Tyr Leu Lys Lys Ile
1 5 10
Lys Asn Ser Ile Ser
15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

His Ile Glu Gln Tyr Leu Lys Lys Ile Lys Asn Ser Ile
1 5 10
Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Plasmodium falciparum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly Asp Ile Glu Lys Lys Ile Ala Lys Met Glu Lys Ala
1               5                   10
Ser Ser Val Phe Asn Val Val Asn Ser
    15                  20
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala Ser Val
1 5 10
Asn Cys Ala
15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn
1 5 10
Glu

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Tyr Thr Tyr Thr Val His Ala Ala His Ala Tyr Thr Tyr
1               5                   10
Thr
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Measles virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Ile Leu Glu Ser Arg Gly Ile Lys Ala Arg Ile Thr
1 5 10
His Val Asp Thr Glu Ser Tyr
15 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Measles virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Leu Ser Glu Ile Lys Gly Val Ile Val His Arg Leu Glu
1 5 10
Gly Val
15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Respiratory syncytial virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Cys Glu Tyr Asn Val Phe His Asn Lys Thr Phe Glu Leu
1               5                   10
Pro Arg Ala
    15
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Influenza virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Ser Phe Glu Arg Phe Glu Ile Phe Pro Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Influenza virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Val Thr Ala Ala Cys Ser His Glu Gly Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Influenza virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Cys Pro Lys Tyr Val Arg Ser Ala Lys Leu Arg Met
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: pig

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: pig

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis B virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Gln Trp Asn Ser Thr Thr Phe His Gln Thr Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis B virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly
1 5 10
Gln Asn
15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis B virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys
1 5 10
Pro Leu
15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Hepatitis B virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile
1               5                   10
Pro Ser
    15
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Foot and mouth disease virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Val Pro Asn Leu Arg Gly Asp Leu Gln Val Leu Ala Gln
1 5 10
Lys Val Ala Arg Thr Leu Pro
15 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rabies virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Glu Gly Cys Thr Asn Leu Ser Gly Phe Ser Tyr Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Plasmodium falciparum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Asn Ala Asn Pro
1

CLAIMS

1. A synthetic peptide T cell epitope analogue of a native T cell epitope which analogue is partially or completely inverso modified with respect to the native T cell epitope.

2. A synthetic peptide T cell epitope analogue of a native T cell epitope which analogue is partially or completely retro-inverso modified with respect to the native T cell epitope.

3. A synthetic peptide T cell epitope analogue acoording to claim 1 or claim 2 wherein the native T cell epitope is selected from the group consisting of:
H-Gln-Val-Val-His-Asn-Ser-Tyr-Asn-Arg-Pro-Ala-Tyr-Ser-Pro-Gly-OH, from diphtheria toxin (SEQ ID NO: 1);
H-His-Arg-Met-Gln-Glu-Ala-Val-Glu-Ala-Glu-Arg-Ala-Gly-Arg-OH, from pertussis toxin (SEQ ID NO: 2);
H-Pro-Ser-Asp-Lys-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-OH, from malaria CSA protein (SEQ ID NO: 3);
H-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-OH, from malaria CSB protein (SEQ ID NO: 4);
H-Gly-Asp-Ile-Glu-Lys-Lys-Ile-Ala-Lys-Met-Glu-Lys-Ala-Ser-Ser-Val-Phe-Asn-Val-Val-Asn-Ser-OH, from malaria CST3 protein (SEQ ID NO: 5);
H-Cys-Ser-Ala-Leu-Leu-Ser-Ser-Asp-Ile-Thr-Ala-Ser-Val-Asn-Cys-Ala-OH, from hen egg lysozyme (SEQ ID NO: 6);
H-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-OH (SEQ ID nO: 7) and
H-Tyr-Thr-Tyr-Thr-Val-His-Ala-Ala-His-Ala-Tyr-Thr-Tyr-Thr-OH (SEQ ID NO: 8), from ovalbumin;
MVF:258-277 H-Gly-Ile-Leu-Glu-Ser-Arg-Gly-Ile-Lys-Ala-Arg-Ile-Thr-His-Val-Asp-Thr-Glu-Ser-Tyr-OH (SEQ ID NO: 9)
and
MVF:288-302 H-Leu-Ser-Glu-Ile-Lys-Gly-Val-Ile-Val-His-Arg-Leu-Glu-Gly-Val-OH (SEQ ID NO: 10), from measles virus F and H glycoproteins;

RS1A:45-60 H-Cys-Glu-Tyr-Asn-Val-Phe-His-Asn-Lys-Thr-Phe-Glu-Leu-Pro-Arg-Ala-OH (SEQ ID NO: 11), from respiratory syncytial virus 1A protein;

Influenza hamagglutinin A/PR/8/34 Mt.S.: residues 109-119 (SEQ ID NO: 12), 130-140 (SEQ ID NO: 13), and 302-313 (SEQ ID NO: 14);

residues (A)4-14 (SEQ ID NO: 15) and (B)5-16 (SEQ ID NO: 16) from pork insulin;

Hepatitis B virus pre S residues 120-132 (SEQ ID NO: 17);

Hepatitis B virus major surface antigen: residues 38-52 (SEQ ID NO: 18), 95-109 (SEQ ID NO: 19), and 140-154 (SEQ ID NO: 20);

Foot and mouth virus VP1: residues 141-160 (SEQ ID NO: 21); and

Rabies virus-spike glycoprotein precursor: residues 32-44 (SEQ ID NO: 22).

4. A T cell epitope analogue according to claim 2 or claim 3 wherein the amino acid residues flanking the retro-inverted sequence are substituted by side-chain-analogous α-substituted geminal-diaminomethanes and malonates.

5. A vaccine comprising a T cell epitope analogue according to any one of claims 1 to 4 together with a B cell epitope and a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

6. A vaccine according to claim 5 wherein the T cell epitope analogue is conjugated to the B cell epitope.

7. A vaccine according to claim 5 which is a cocktail of T cell epitope analogues and B cell epitopes tailored to the condition against which vaccination is required.

8. A vaccine according to claim 5 wherein the B cell epitope is a peptide or polypeptide of any length whose amino acid sequences stem from:

polypeptides of a pathogen including poliomyelitis, hepatitis B, foot and mouth disease of livestock, tetanus, pertussis, HIV, cholera, malaria, influenza,

rabies or diphtheria causing agents;
a toxin including robustoxin, heat labile toxin of pathogenic *Escherichia coli* strains and Shiga toxin from *Shigella dysenteriae;*
Amyloid ß protein;
human chorionic gonadotropin;
or gonadotropin releasing hormone.

9. A vaccine according to claim 5 wherein the B cell epitope is a retro, retro-inverso or inverso antigen analogue.

10. A method of vaccinating a host in need of such treatment which method comprises administering an effective amount of a vaccine according to claim 5 to the host.

11. A method of preparing a T cell epitope analogue according to claim 1 or 2, the method comprising synthesising a partially or completely inverso or retro-inverso analogue of the native T cell epitope.

12. Antibodies produced by immunisation of a host with a vaccine according to claim 5.

13. A method of preparing a vaccine according to claim 5 which method comprises: conjugating a T cell epitope analogue according to claim 1 or claim 2 to a B cell epitope, or admixing a T cell epitope analogue according to claim 1 or claim 2 with a B cell epitope; and admixing an effective amount of the resulting mixture or conjugate with a pharmaceutically or veterinarally acceptable carrier, diluent, excipient and/or adjuvant.

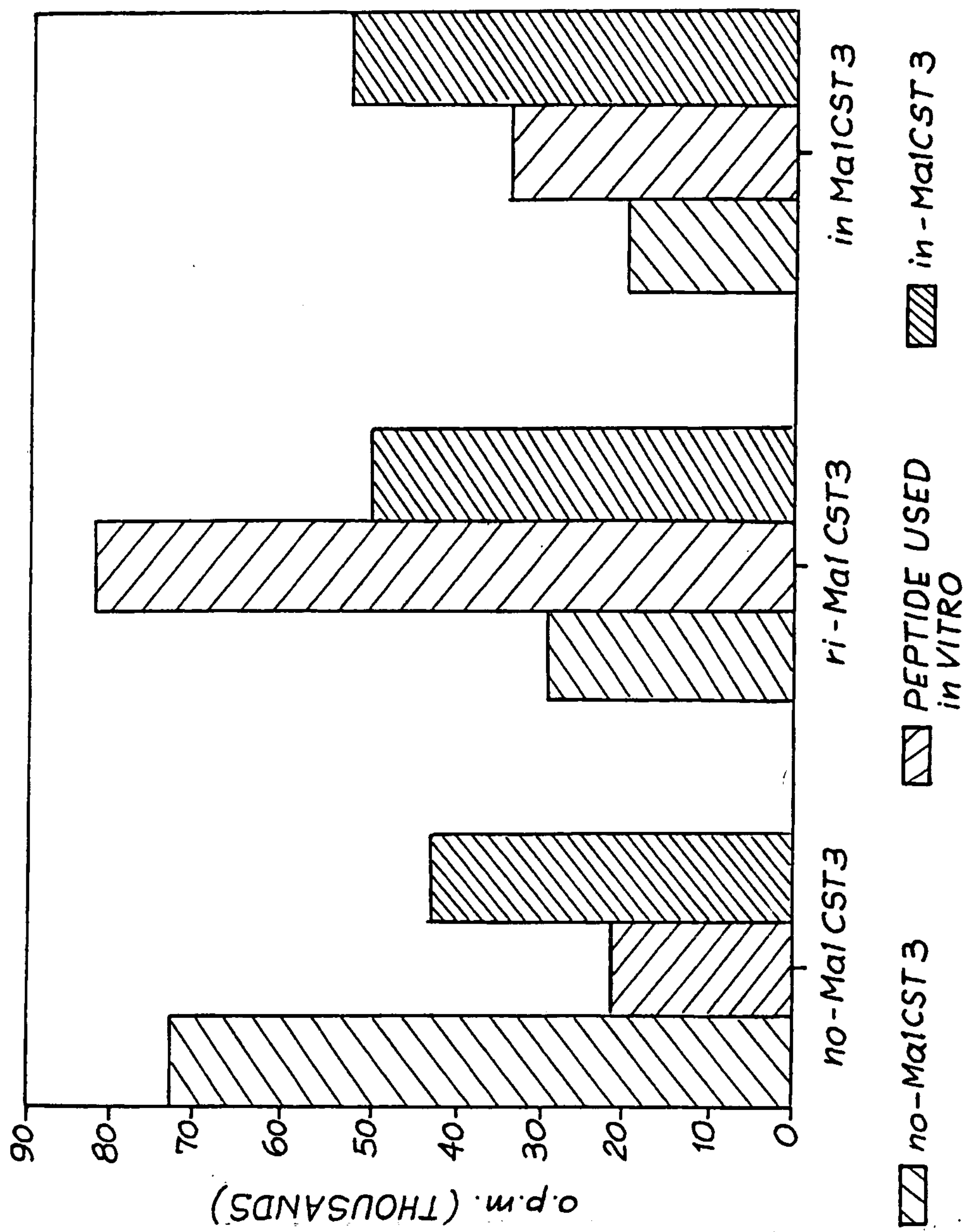
c.p.m. (THOUSANDS)
0
10
20
30
40
50
60
70
80
90
no-Mal CST3
ri-Mal CST3
in Mal CST3
PEPTIDE USED
in VITRO
no-MalCST3
ri-MalCST3
in-MalCST3

FIG. 1

1/TITRE	CONTROL ■	NORMAL □	ANALOGUE ◆
2	0.84	1.425	1.32
4	0.555	1.89	1.585
8	0.465	1.825	1.775
16	0.24	0.84	1.16
32	0.265	0.9	0.935
64	0.08	0.74	0.815
128	0.09	0.625	0.555
256	0.06	0.305	0.335
512	0.02	0.23	0.14
1024	0	0.095	0.07
2048	0.005	0.095	0.06

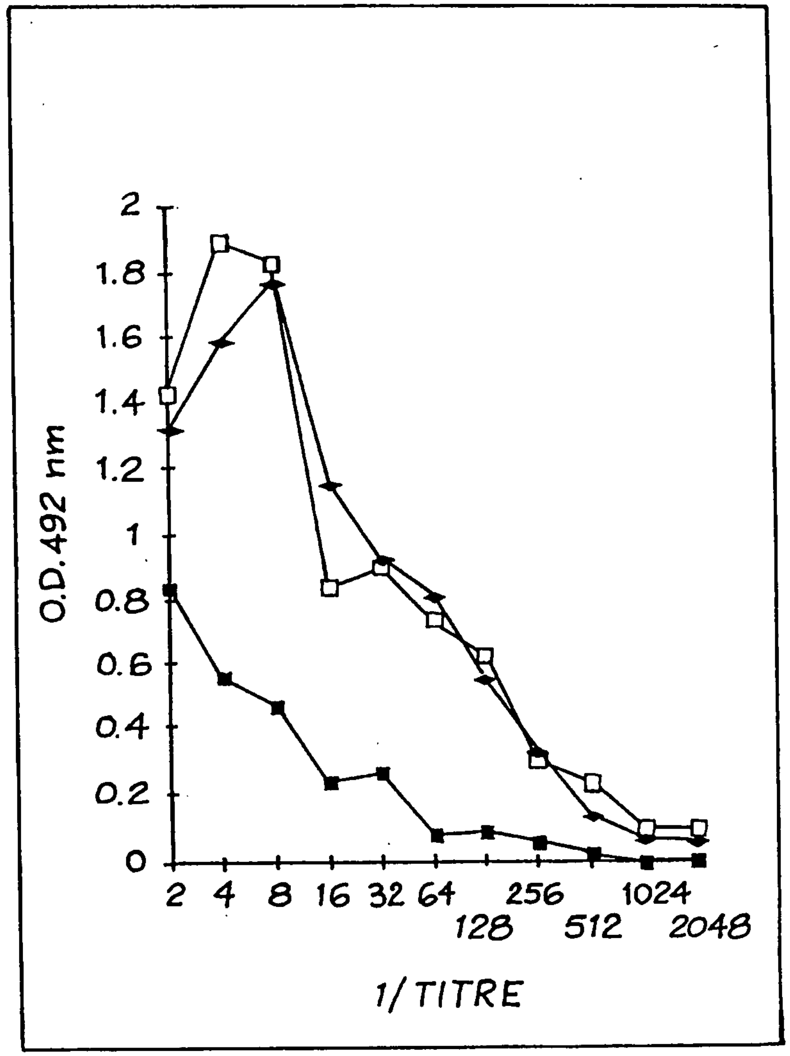

FIG. 2

1/TITRE	CONTROL ■	NORMAL □	ANALOGUE ◆
5	0.733	1.343	2.667
10	0.651	0.98	2.317
20	0.481	0.726	1.266
40	0.266	0.551	1.231
80	0.232	0.42	0.712
160	0.126	0.277	0.612
320	0.123	0.194	0.561
640	0.071	0.11	0.456
1280	0.061	0.104	0.317
2560	0.026	0.102	0.231
5120	0.023	0.101	0.126

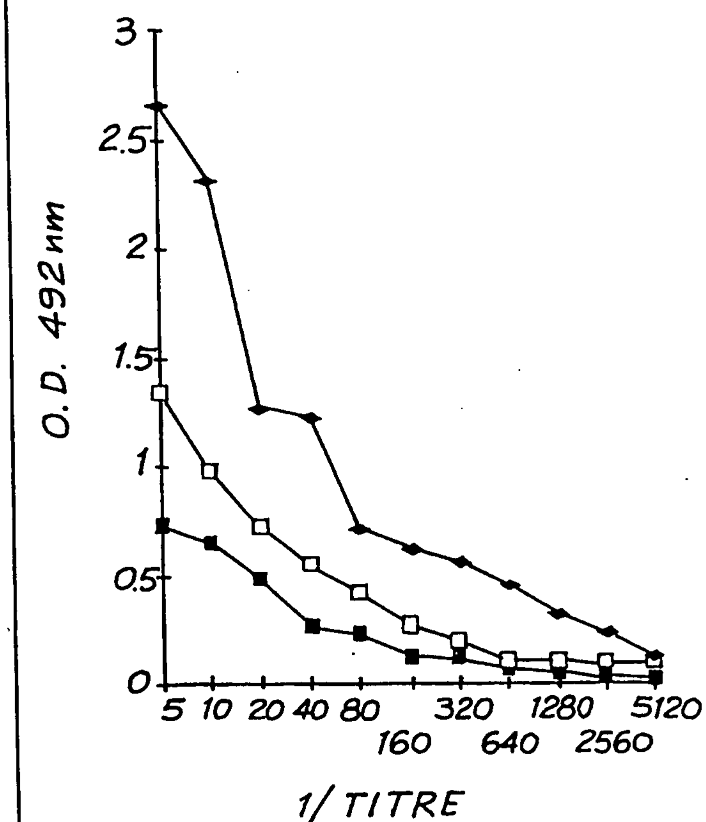

FIG. 3

1/TITRE	CONTROL ■	NORMAL □	ANALOGUE ◆
5	0.806	1.567	2.96
10	0.2	1.252	2.7
20	0.175	0.692	1.534
40	0.06	0.682	0.951
80	0.056	0.495	0.854
160	0.041	0.697	0.629
320	0.035	0.239	0.54
640	0.017	0.095	0.44
1280	0.006	0.072	0.35
2560	0.005	0.061	0.29
5120	0.001	0.027	0.164

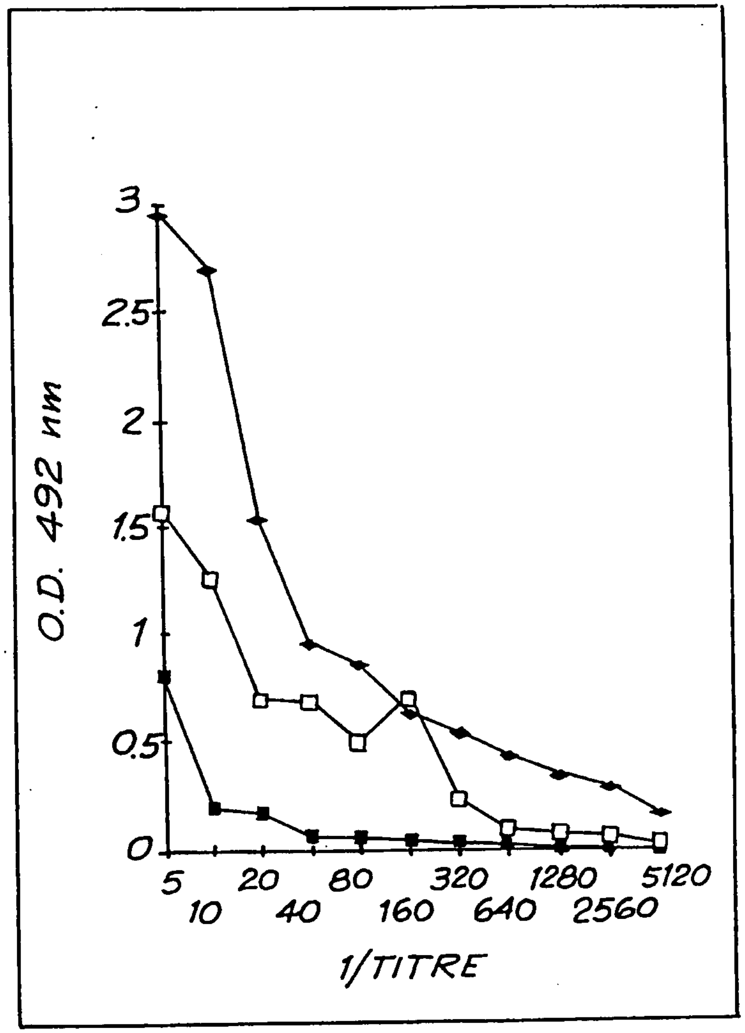

FIG. 4

1/TITRE	CONTROL ■	NORMAL □	ANALOGUE ◆
5	0.967	2.63	2.887
10	0.821	1.76	2.438
20	0.66	1.65	2.335
40	0.416	0.832	1.86
80	0.411	0.775	1.364
160	0.284	0.727	0.934
320	0.242	0.619	0.795
640	0.193	0.434	0.652
1280	0.109	0.2	0.53
2560	0.098	0.074	0.38
5120	0.097	0.069	0.177

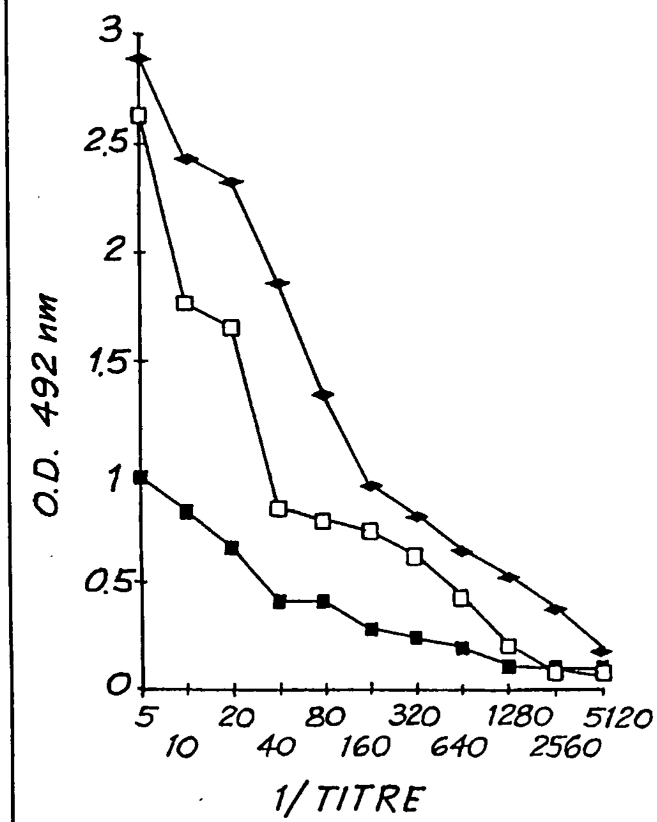

FIG. 5

1/TITRE	CONTROL ■	NORMAL □	ANALOGUE ◆
5	0.868	2.823	1.354
10	0.85	1.395	1.363
20	0.837	1.351	1.284
40	0.65	1.425	0.844
80	0.38	0.775	0.615
160	0.271	0.643	0.554
320	0.266	0.603	0.42
640	0.163	0.378	0.196
1280	0.084	0.271	0.253
2560	0.076	0.211	0.157
5120	0.073	0.192	0.096

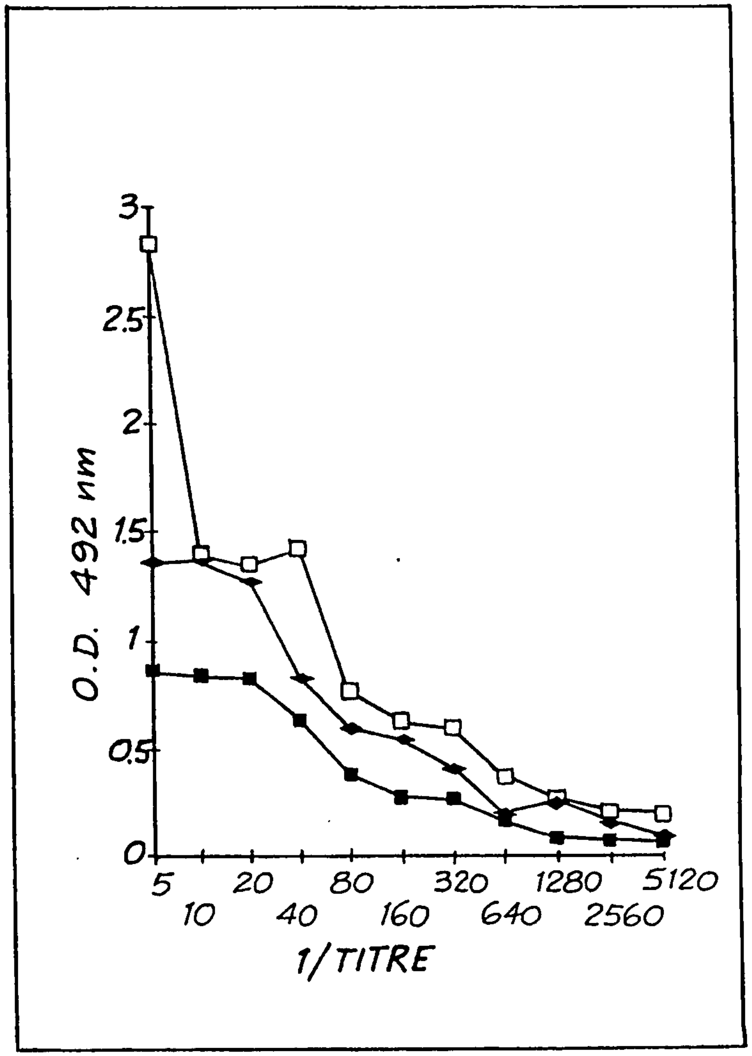

FIG. 6

1/TITRE	CONTROL ■	NORMAL □	ANALOGUE ◆
5	0.991	2.375	2.646
10	0.51	1.473	1.373
20	0.351	1.217	0.832
40	0.11	0.663	0.645
80	0.092	0.595	0.644
160	0.054	0.186	0.412
320	0.026	0.158	0.214
640	0.023	0.149	0.191
1280	0.006	0.087	0.133
2560	0.005	0.072	0.087
5120	0.003	0.071	0.074

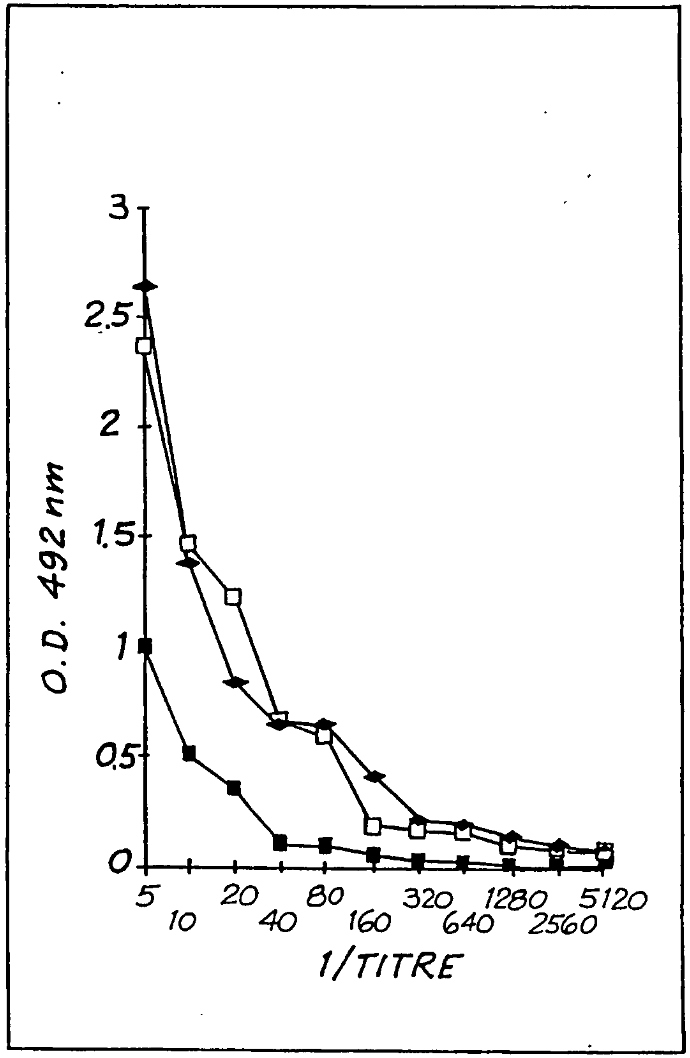

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 95/00090

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.^{6}
C07K14/705,14/34,14/235,14/445,14/165,14/77,14/12,14/135,14/11,14/62,14/02,14/09,14/145,16/28,16/08,16/10,16/12,16/18,16/20,16/26; A61K 39/00,39/015,39/05,39/10,39/145,39/135,39/165,39/205,39/29,39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: as above using Derwent (WPAT) and keywords.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU:IPC: as above.

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
Derwent (WPAT): Retro or Inverso or Retro( ) Inverso and T-cell
Chemical Abstracts: Retro or Inverso or Retro ( ) Inverso and not Retrovirus or Retroviral or Retrobulbar and T-cell

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	AU 49346/93 A (DEAKIN RESEARCH LIMITED) 29 March 1994 whole document, especially Examples 6, 7, 14-17, Claims 7-9,12	1-4,12
A	ANGEWANDTE CHEMIE Vol. 31, No. 6 (1992) DUERR, Hansjoerg, et al. 'Retro-inverso amide bonding between trifunctional amino acids' whole document	1-4

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 1995	5 JUNE 1995 (05. 06. 95)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929	Authorized officer JENNIFER POTTER Telephone No. (06) 2832247